REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claims 1-16 and 21-22 have been amended in this paper. Therefore, claims 1-30 are pending. Of these claims, claims 17-20 and 23-30 are withdrawn as being drawn to a non-elected invention or a non-elected species. Therefore, claims 1-16 and 21-22 are under active consideration.

In the outstanding Office Action, the Patent Office states in paragraph 5 that "Applicant did not define the term 'activation-induced cytidine deaminease-AID', therefore any cytidine deaminase is considered to anticipate this term."

Applicant respectfully traverses the foregoing position of the Patent Office. Applicant respectfully submits that the term "activation-induced cytidine deaminase (AID)" is the name of a specific cytosine deaminase that is well-known by a person of ordinary skill in the art (see, for example, the first paragraph of page 4 of the present specification, which refers to Storb and Stavnezer: Immunoglobulin Genes: Generating Diversity with AID and UNG, Curr Biol. 2002 Oct. 29; 12(21):R725-7, as well as page 14 of the present specification, which refers to Bransteitter et al., PNAS, v. 100, p. 4102 (2003), and Pham Phuong et al., "Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation, Nature, 424(6944):103-7 (2003), which was cited in the International Search Report). Moreover, Applicant specifically states the following on page 3 of the present specification: "The invention which is disclosed here is based on the application of cytidine deaminases, which convert cytidine and 5-methylcytidine at different

rates. It particularly involves *activation-induced cytidine deaminase* – AID." Consequently, Applicant has clearly distinguished the class of cytidine deaminases from the specific enzyme AID, and there is no basis for the Patent Office's position that any cytidine deaminase is considered to be AID. In fact, Applicant respectfully submits that such a position runs directly contrary to the specific teachings of the present specification.

Claim 2 stands rejected under 35 U.S.C. 112, first paragraph, "as failing to comply with the written description requirement" as it is alleged that "[t]he claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." As best understood, the rejection appears to be predicated on the position that the claim encompasses "a genus of cytidine deaminases which have not been described" and "variants for which no written description is provided in the specification" and that "[t]his large genus is represented in the specification by only the particularly named cytidine deaminase, called AID by Applicant, and characterized by citations from different references" but that "no specific sequence encoding the AID protein or its amino acid sequence were provided."

Applicant respectfully traverses the subject rejection. For reasons similar to those given above, Applicant respectfully submits that a person of ordinary skill in the art, at the time of the present invention, would have understood what is meant by AID and would have been in possession of whatever information is needed to describe, to make, and to use AID. In fact, Applicant points out in the paragraph bridging pages 4 and 5 of the present specification that the nucleic acid and amino

acid sequences of AID are disclosed in European Patent Application Publication No. 1,174,509 (which was published January 23, 2002).

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-16, 20 and 21 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claims are written in the passive tense. Method claims should recite positive, active process steps (see Ex parte Erlich 3 USPQ 2d 1011). This rejection may be overcome by amending the claims to recite the active tense, e.g., "bringing the DNA into contact with cytidine deaminase," etc.

Without acquiescing in the propriety of the rejection, the claims have been amended to use the active voice. Accordingly, the rejection has been obviated and should be withdrawn.

Claims 1-13 stand rejected under 35 U.S.C. 102(a) "as being anticipated by Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS)." In support of the rejection, the Patent Office states the following:

> Regarding claim 1, Bransteitter et al. teach a method for the detection of cytosine methylations in DNA (abstract) characterized in that
> a) the DNA to be investigated is brought into contact with a cytidine deaminase, whereby the cytidine deaminase deaminates cytidine and 5-methylcytidine at different rates (page 4102, paragraphs 3-5),
> b) the partially deaminated DNA is investigated with respect to its sequence (page 4102, last paragraph; page 4103, first and second paragraph), and
> c) from the presence or the proportion of deaminated postions, conclusions can be made on the methylation status of the DNA to be investigated in said positions (Fig. 1; Fig. 2).
>
> Regarding claim 2, Bransteitter et al. teach AID (page 4102, fourth paragraph).

> Regarding claims 3 and 4, Bransteitter et al. teach single-stranded and partially-single stranded DNA (page 4102, third paragraph; Table 1).
>
> Regarding claims 5-7, Bransteitter et al. teach single stranded regions being between 3 and 20 nucleotides long, between 5 and 12 nucleotides long and 9 nucleotides long (Table 1, page 4106).
>
> Regarding claims 8 and 9, Bransteitter et al. teach oligomers between the length of 20 to 150 nucleotides and 35-60 nucleotides (Table 1, page 4106).
>
> Regarding claims 10 and 11, Bransteitter et al. teach oligomers concentration of 100 nM (page 4102, fifth paragraph), anticipating the claimed ranges.
>
> Regarding claims 12 and 13, Bransteitter et al. teach amplification of the deaminated fragment using a polymerase (page 4103, second paragraph).

Applicant respectfully traverses the subject rejection. Bransteitter et al. does not anticipate claims 1-13 for at least the reason that Bransteitter et al. does not teach, amongst other things, step (c) of the claimed method, namely, concluding, from the presence or the proportion of deaminated positions, the methylation status of the DNA to be investigated in said positions. The Patent Office is apparently taking the position that step (c) is taught by Figs. 1 and 2 of Bransteitter et al. Applicant respectfully disagrees. Figs. 1a and b refer to assay 1 while Fig. 1c refers to assay 2 (see description for Fig. 1). The paragraph bridging pages 4102 and 4103, as well as the description for Fig. 1, describes assay 1. According to it, (a) oligonucleotides are 5'end-labeled; (b) ssDNA is incubated with AID; (c) a complementary ssDNA is annealed to the AID treated ssDNA; (d) the product of step (c) is incubated with UDG and APG; and (e) a nick at an original dC site is detected wherein an AID catalyzed dC-dU conversion is visualized. See also Fig. 1a. In contrast to the

claimed method, neither assay 1, Fig. 1a or 1b, nor the description of Fig. 1 makes any statement about the methylation status of the analyzed DNA.

The same applies to assay 2. A description for assay 2 can be found on page 4103, left column, last paragraph, and in the description for Fig. 1. According to it, (a) AID treated ssDNA was annealed to a primer; (b) the annealed primer is elongated by means of a mixture of three dNTPs and either ddA or ddG; (c) the so-formed dsDNA substrates were denatured and annealed to primer that are complementary to the original applied AID treated dsDNA; (d) products of step (c) were subjected to denaturing gel electrophoresis and phosphorimaging; and (e) deamination efficiencies of extension reactions were calculated from band intensities. Deamination of ssRNA was analyzed correspondingly with the exception of using an RNA-polymerase for extension (see also Fig. 2 and its description). However, in contrast to the claimed method, assay 2 and Fig. 1c are silent about making conclusions on the methylation status of the analyzed DNA.

In addition, Fig. 2 of Bransteitter et al. refers to the finding that RNase activates AID by digesting AID-associated inhibitor RNA. For this, AID is pre-treated with RNase before application into assay 2 (description for Fig. 2). Also here, Bransteitter et al. is silent about the methylation status of analyzed DNA as it is the subject matter of claim 1, step c).

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 12-16, 21 and 22 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107), April 2003; cited in the IDS) and Olek et al. (U.S. Patent No. 7,229,759 B2)." In support of the rejection, the Patent Office states the following:

A) Bransteitter et al. teach detection of the converted uracil residues using primer extension and ddA, but do not teach PCR or real-time PCR or using blocker oligonucleotides in the amplification reaction.

B) Regarding claims 12-14, 21 and 22, Olek et al. teach detection of deaminated cytosines resulting from bisulfite reaction using real-time PCR (col. 5, lines 37-53; col. 13, lines 45-59).

Regarding claim 15, Olek et al. teach methylation-specific primers (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31).

Regarding claim 16, Olek et al. teach using blocking oligonucleotides during amplification (col. 6, lines 3-20 and 38-67; col. 11, lines 29-49).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used the amplification methods of Olek et al. with blocking oligonucleotides to detect the converted cytidines in the method of Bransteitter et al. The motivation to do so is provided by Olek et al. (col. 13, lines 52-59 and col. 11, lines 67 and col. 12, lines 1-3):

"A particularly preferred variant of the method, however, is the simultaneous detection of qualifier positions and classifier positions in one experiment, which can be achieved by the use of TaqMan or LightCycler technology variants. Additionally fluorescently labeled oligonucleotides are to be added to the oligonucleotides, which provide for a preferred amplification of the DNA to be investigated, and the change in fluorescence is measured during the PCR reaction. In principle, since the DNA to be investigated is amplified, information on the methylation status of different classifier CpG positions is obtained predominantly also directly from this change in fluorescence. Since different oligonucleotides are each preferably provided with different fluorescent dyes, a distinction of the change in fluorescence during the PCR is also possible, separately for different positions."

"If only one small group of CpGs is available and still a high amount of background DNA has to be blocked, it is therefore preferred that one part of this group of CpGs is covered by a methylation specific primer and the other part is covered by a methylation specific blocking probe, and the binding site of this non-extendible probe could ideally even overlap with the binding site of

the primer. This way, high relative sensitivity, this means highly preferred amplification of the DNA to be analyzed while suppressing the background DNA, can be achieved with only a small group of CpGs as Qualifier positions."

Applicant respectfully traverses the subject rejection and respectfully submits that the combination of Bransteitter et al. and Olek et al. teaches away from the claimed invention.

As the Patent Office correctly notes, Bransteitter et al. teaches the detection of deaminated cytosines, i.e., converted uracils using primer extension and a dNTP mixture of three nucleotides additionally comprising either ddA or ddG (assay 2 - p4103, left column, last paragraph). The method of Bransteitter et al. comprises (a) annealing AID treated ssDNA to a primer; (b) elongating the annealed primer by means of a polymerase and three dNTPs and either ddA or ddG; (c) denaturing the so-formed dsDNA substrates; (d) annealing primer complementary to the original applied AID treated ssDNA; (e) subjecting products of step (d) to denaturing gel electrophoresis and phosphorimaging; and (f) calculating deamination efficiencies from band intensities. Because Bransteitter explicitly states that deamination efficiencies were calculated from extension reactions with ddA mix or from extension reactions with ddG mix, a person of ordinary skill in the art would have considered the use of such ddA mix or ddG mix as necessary for detecting deamination of cytosines, i.e., converted uracils.

For this reason, even if a person of ordinary skill in the art would have had the idea of combining the method of Bransteitter with the method of Olek, he would have ended up with a method wherein said ddA or said ddG mixes are used for primer extension, irrespective of whether this primer extension is a single extension or part of a PCR, real-time PCR or conducted in the presence of blocking oligonucleotides. However, according to the claimed method, a primer

extension is not necessary. According to step (b) of claim 1, the sequence of partially deaminated DNA is analyzed. According to claim 12 (as well as claims 13-16 dependent therefrom), such analysis may comprise amplification, preferably a polymerase mediated amplification, more preferably by means of PCR, and most preferably either in the presence of methylation-specific primers or a methylation specific blocker oligonucleotide. In contrast to the teaching of Bransteitter and thus in contrast to a combination of Bransteitter and Olek, the use of a mixture of three dNTPs with either ddA or ddG is not necessary.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on November 4, 2009

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